

Open Notebook Science

Speed effects on gliding motility assays due to surface passivation, water isotope, and osmotic stress.

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This work is supported by the DTRA CB Basic Research Program under Grant No. HDTRA1-09-1-008 and the UNM IGERT on Integrating Nanotechnology with Cell Biology and Neuroscience NSF Grant DGE-0549500. We would also like to thank Susan Atlas, PI of the DTRA project and Dr. Haiqing Liu for supplying us kinesin-1.

Surface passivation effects

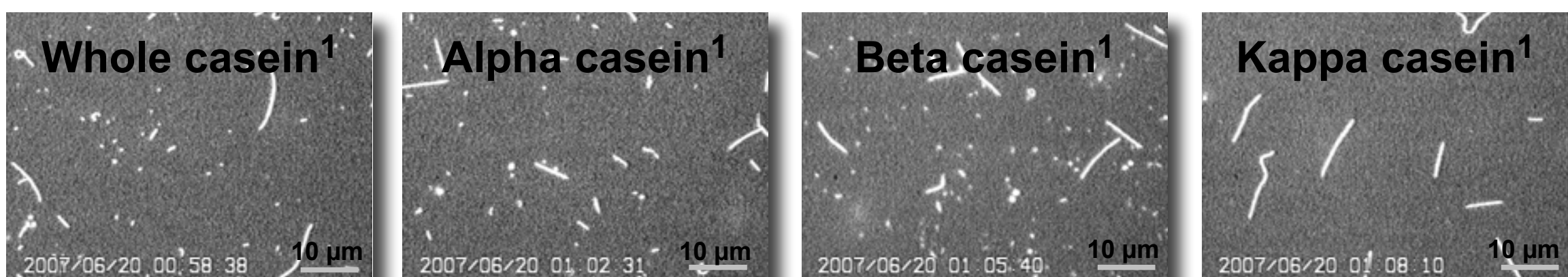
Motivation

For kinesin to function properly in a gliding motility assay, the substrate to which kinesin adheres must first be passivated¹. Functionalization of glass slides and slips is typically performed with bovine milk proteins called caseins, however, BSA can also be used as a passivator⁷. Bovine casein is a globular protein that can be broken up into four major constituents: α_s , α_{s2} , β , and κ . Each casein constituent affects how kinesin adheres to the glass (or possibly how kinesin adheres to casein) and they ultimately affect the speed at which microtubules are observed to glide. Building on the work of Verma et. al.¹, we have found that each constituent of bovine casein produces different outcomes in gliding assays.

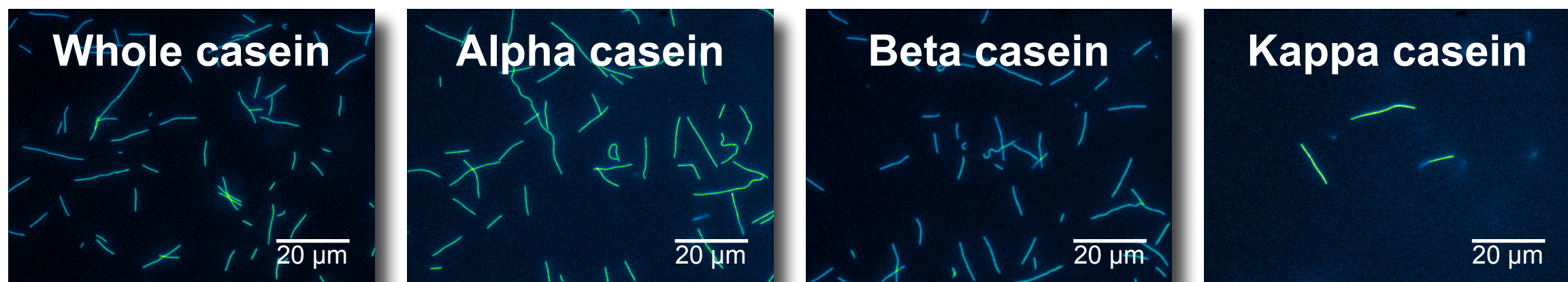
Experiment

All chemicals were purchased from Sigma unless stated otherwise. In order to determine the differences in how kinesin and microtubules interact dependent on casein constituents used as passivators, we first introduced caseins to our base buffer PEM [80mM PIPES (Sigma-80635), 1mM EGTA (Sigma-03778), 1mM MgCl₂ (Sigma-M1028), pH 6.89] at 0.5mg/mL. α casein, (a mixture of α_s ; α_{s2} ~ 7:2, Sigma-C6780), β casein (Sigma-C6905), and κ casein (Sigma-C0406) go into solution easily with constant stirring, however, whole casein is notoriously difficult to get into solution. There exists a great deal of legacy procedures in the community on how to get whole casein into solution, some of which include extended periods of centrifugation and filtration. Whole casein does not have a secondary structure and will not denature under heat³. Thus, to get whole casein into PEM, we heat the solution and stir in 0.5mg/mL whole casein until it is completely dissolved since mild heating cannot affect the casein micelle structure. Finally, all casein solutions are passed through a 0.2 μ m filter to remove any possible bacteria that may be in the solution.

Results

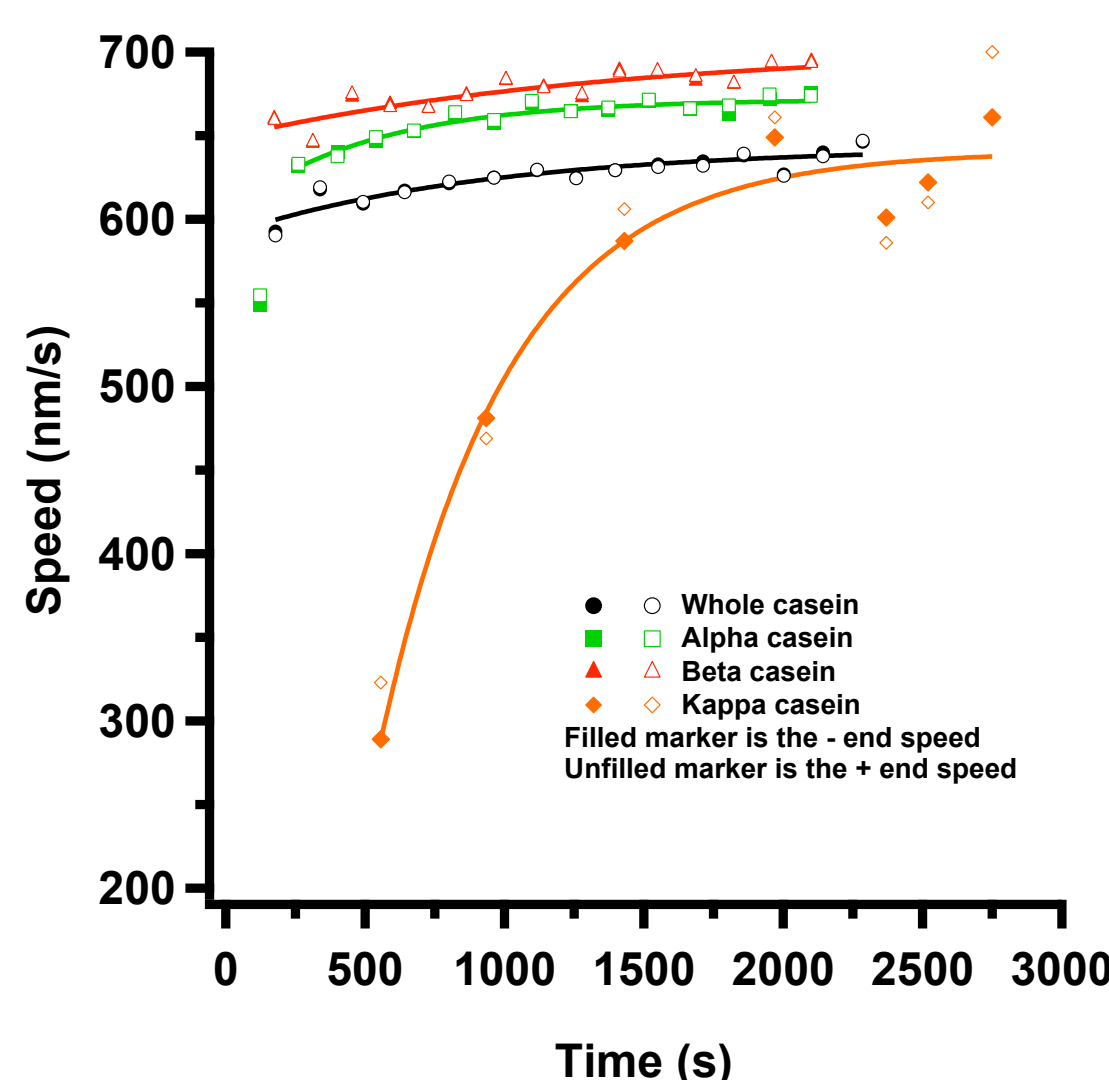


Very nice work done by Verma et. al.¹ (above) shows that microtubules are supported differently in the gliding motility assay dependent on the type of casein used as a passivator. Building on what they have done (below), we investigated the speed changes microtubules glide at depending on what type of passivation is used. Our preliminary work notes that kappa casein is by far the worst at supporting the gliding motility assay at the kinesin concentration we use, 30 μ g/mL. Images are false colored using ImageJ.



Data analysis

Microtubules are tracked using custom LabVIEW software. Please see Larry Herskowitz's poster for a more detailed explanation of the tracking software. Briefly, data is taken from a total of 15 regions in each slide. Data is smoothed using a sliding Gaussian window to eliminate Brownian noise. We then determine the probability density function for instantaneous microtubule speed from a kernel density estimation using a Gaussian kernel of width 50nm/s. The peak of the PDF is used as the speed for that ROI. An interesting feature of the data is that the microtubules speed up over time in all assays. The β casein passivation supports the fastest microtubule gliding speed while the κ casein passivation is the worst at supporting the gliding assay for these conditions. Each ROI, and correspondingly each data point, is illuminated for approximately 2 minutes. The illumination source is a 100W Hg lamp that is attenuated by 94% and sent through a standard TRITC filter cube.



Water isotope effects

Motivation

Water plays an important role in biological systems and it is often overlooked⁴. It is a key component for the hydrolysis of ATP and has a crucial role in how proteins interact with one another due to the hydration of their surfaces. To the best of our knowledge, no work has been done on investigating how the kinesin-1 microtubule system changes when changes with water isotopes occur. Changing the water isotopes from hydrogen to deuterium or ¹⁶O to ¹⁸O may affect how kinesin interacts with microtubules. This change can be used as a novel experimental knob to probe features of the kinesin and microtubule system in new and different ways.

Experiment

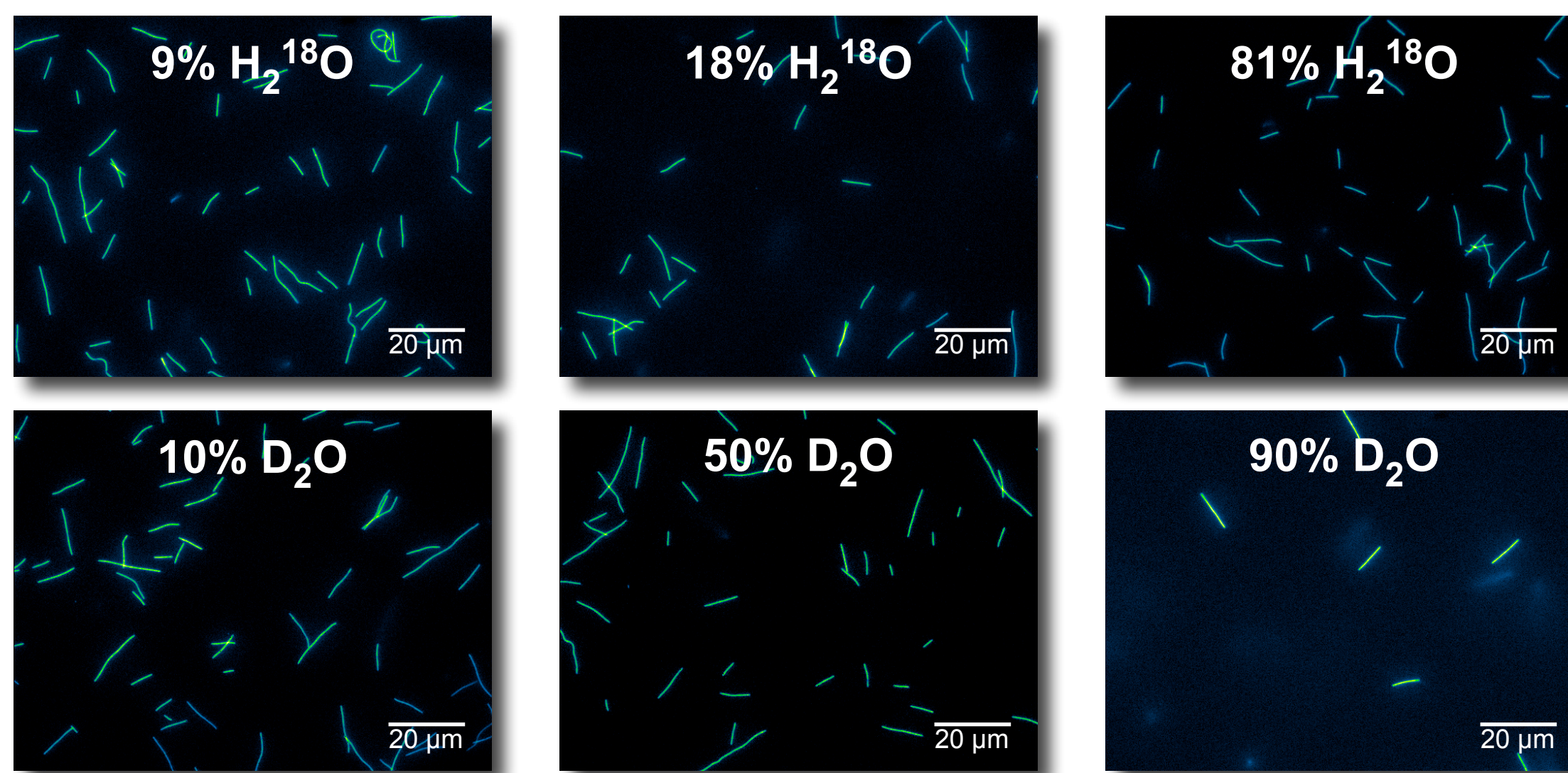
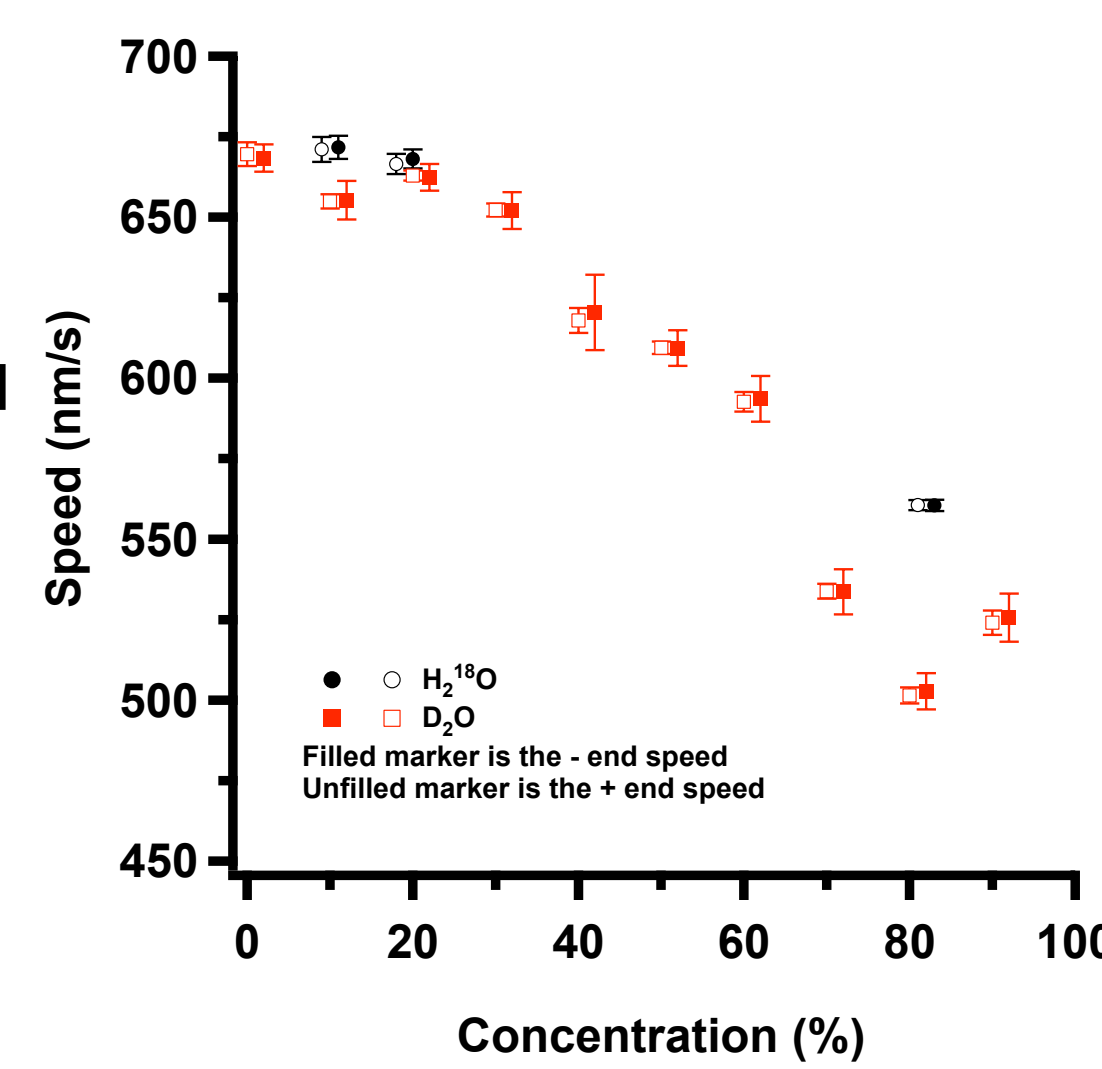
All chemicals were purchased from Sigma unless noted otherwise. Slide preparation was performed as follows:

- 10 minute incubation of slide with 0.5mg/mL α casein in PEM.
- 5 minute incubation of slide with 0.25mg/mL α casein, 30 μ g/mL kinesin-1, and 1mM ATP in PEM.
- Introduction of Taxol stabilized microtubules with an enzymatic antifade system and 1mM ATP in PEM with the desired mix of water isotopes. After sealing, observations are conducted immediately.

When using D₂O, care must be taken in order to measure the correct "pH" denoted as the pD = pH + 0.41⁵. Also, all solutions that have Taxol in them are always prepared fresh and are discarded after 3 hours to prevent Taxol crystals from forming⁶.

Results

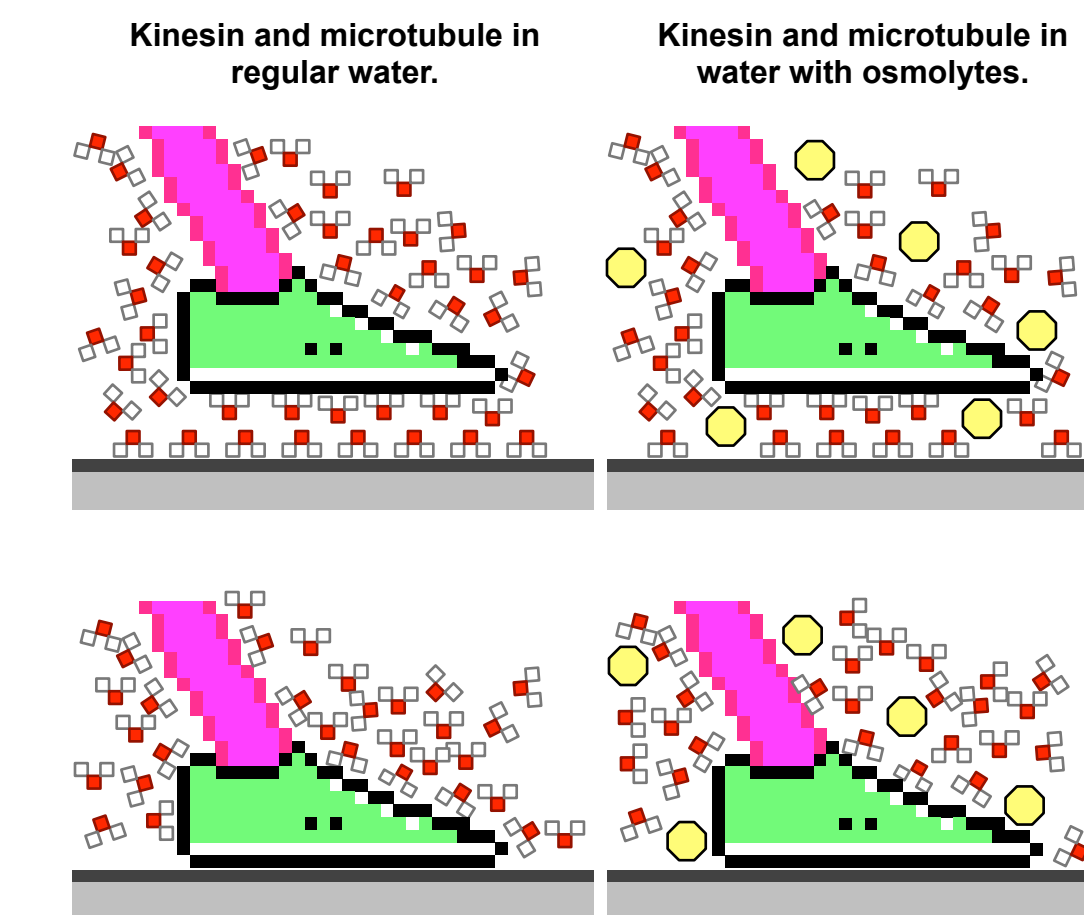
- The tracking software is capable of tracking both the positive end (unfilled markers) and the minus end (filled markers) of the microtubule. Plus and minus ends are offset for clarity in the plot. We use the two tracked numbers as a check to ensure that the microtubules are tracked properly. See Larry Herskowitz's poster for a more detailed description of the tracking software.
- H₂¹⁸O (Sigma-329878, black markers, 97% ¹⁸O) is quite expensive. We purchase this water 1 gram at a time. In order to prepare the PEM solution with H₂¹⁸O water, a 10x solution of PEM is prepared in regular H₂O. It is then diluted by a factor of 10 in H₂¹⁸O. What is plotted above is the % concentration of H₂¹⁸O used in an assay and not the % concentration of ¹⁸O in solution.
- While time prevented us from completing the experiment, You can clearly see the reduced speed trend by increasing H₂¹⁸O concentration in the assay.
- D₂O (Sigma-151882, red markers, 99.9% D) is not as expensive as H₂¹⁸O and so PEM is prepared in D₂O. Again, what is plotted is the % D₂O used in the assay and not the % deuterium in solution.
- A distinctive trend is observed as one reduces the amount of H₂O in the assay.
- Data points at 70% and 80% D₂O may be an experimental error and thus may not represent the true nature of the system.
- Error bars are the SEM of 8 ROIs where the slight increase in speed over the 8 regions is ignored (see data in the surface passivation section).



Osmotic stress effects

Motivation

During the stepping process, the kinesin motor domains bind and unbind from their binding sites on the microtubules. Binding and unbinding rates of biomolecules are highly dependent on hydration and exclusion of water from the binding interface. Osmotic stress will likely strongly affect the binding and unbinding rates for kinesin and thus may offer a tool to specifically probe those steps.



Experiment

- All chemicals were purchased from Sigma unless noted otherwise. Slide preparation was done exactly as stated in the Experiment part of Water isotope effects. We chose to use α casein as the surface passivator since it showed the closest speed similarities to whole casein which is the passivator most people use.
- A stock solution of 5M Betaine (Sigma-61962) in PEM was prepared and diluted into the motility solution for the experiments.
- Future work will include how motility is affected with the addition of other known osmolytes: Sarcosine (Sigma-S7672), D-Proline (Sigma-858919), TMAO (Sigma-317594), and other chemicals.
- In order to determine if osmotic stress is the key descriptor as to why gliding speeds are changed, viscosity measurements must be done on the assay solutions. Betaine is known to have less increase in viscosity than sucrose for example, however, viscosity remains a confounding issue that we need to explore.

Results

- Even the 0mM Betaine data set has a significant osmotic pressure relative to regular water due to the osmotic agents present in the standard motility solution.
- The antifade system constitutes a portion of the initial osmotic pressure since it contains glucose oxidase (Sigma-G6641), catalase (Sigma-C9322), BME (Sigma-63689), and 20mM glucose (Sigma-49139).
- Initial studies show that gliding speed is affected by osmotic stress or viscosity. We are still unsure as to which of these is the strongest affect. Studies⁸⁻¹¹ have been done on myosin in osmotic or viscous stress, however, no consensus has been made as to what the actual affect is coming from.

Discussion

When preparing the samples for the betaine tests, an interesting observation was made with the slide preparations. As can be seen in the images to the left, microtubules for the most part are straight, except for the wiggles which we believe is due to the osmotic stress affect. When preparing these slides, no casein was included in the motility solution that contained the microtubules.

When casein was added to the motility solution, odd squiggle patterns were seen in the assay. This observation led us to not include casein in our motility assay despite literature recommendations¹. Casein was added in the motility solution for the surface passivation studies since those assays were performed before this observation was made.

Acknowledgements

We would like to thank the following people for their extensive help in this project:

- Dr. Haiqing Liu at CINT for supplying the kinesin-1.
- Dr. Susan Atlas PI of the DTRA project.
- OpenWetWare for hosting Open Notebook Science.
- LabVIEW for continued support.
- Anthony Salvagno for insight with the cartoons.

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